Journal of Agriculture and Environment for International Development - JAEID 2020, 114 (2): 7– 20
DOI: 10.12895/jaeid.20202.1179

Phenological and pomological characterization of *Argania spinosa* (L.) to select promising trees for breeding program

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Submitted on: 2020, February 7; accepted on 2020, October 14. Section: Research Papers.

Abstract: The agro-morphological characterization is crucial in order to provide information for the plant breeding programs. The goal of the present study is to determine phenotypic diversity and to detect elite trees. Six hundred eighty individuals of argan tree (*Argania spinosa (L,) Skeels*) were characterized by using sixteen qualitative and quantitative agro-morphological traits. Majority studied characteristics were showing a high degree of variability, but it was highly pronounced for fruit-spine, branches number, trunk diameter and first leaf height. A significant correlation was found among some studied variables. The principal component analysis for quantitative traits showed that three components explained 54% of the total variation and revealed that traits related to fruits, leaves, crown diameter and trunk diameter contributes for a large proportion of the observed variability. The results indicated that most of trees can be considered a good gene pool for breeding programs and rootstock development programs. More, they could be useful as a parent to be crossed to generating appropriate populations or improve cultivars.

Keywords: Argania spinosa, breeding, morphological traits, promising trees

Introduction

Moroccan flora, with 4200 taxa including 1282 subspecies (Valdés *et al*., 2002), is one of the most diverse in the Mediterranean region and the richest in North Africa (Sauvage, 1952). This vast number of vascular plants with an immense percentage of endemic species places Morocco in an important position among other Mediterranean countries (Médail and Quézel, 1997). This important plant diversity is related to the geographical position of Morocco, its varied topography, geology, ecoregion and climate (Rankou *et al*., 2013). Argan tree (*Argania spinosa* (L.) Skeels) extends over 8700 square kilometers (HCEFLCD, 1996) is the only member of the tropical family Sapotaceae in the region (White, 1983) and the unique representative of monospecific genus argania (Pennington, 1991). Argan is an important multipurpose tree, which plays important ecological and socio-economic role, and it is the principal source of income for local population in the rural regions (M'Hirit *et al*., 1998). The products of this species are used in various domains, the oil extracted from the seeds has a high nutritional value and a particular taste, it is part of the human food chain, cosmetics and pharmacology. The leaves, flowers, fruit pulp and press cake are also used in cosmetics pharmacology and livestock feed. This tree helps to fight against desertification and erosion while producing wood for various purposes (Chimi *et al*., 1994; Lybbert *et al*., 2010). In fact, in order to protect argan groves from the overexploitation, it was classified in 1998 as a "Biosphere reserve" by UNESCO, and many efforts are being undertaken for its rehabilitation.

Genetic diversity is the basis of evolutionary potential of species, breeding program and conservation of genetic resources (Wu *et al*., 1999). Genetic diversity is mainly thought of as the amount of genetic variability among individuals or population of a species (Brown, 1983). It results from some genetic distinction between individuals and can be manifest through differences in DNA sequences, in biochemical characteristics (e,g, in protein structure or isoenzyme properties), in physiological properties (e,g, abiotic stress resistance or growth rate) or in morphological characters such as flower color or plant form (Ramanatha and Hodgkin, 2002). The examination of morphological characters has been one of the most ancient classifications of plants and it is indirectly carried to estimate of genetic diversity. Some of them, such as leaves, branches, bud, or traits like viability, resistance to cold and pests and properties of wood are considered crucial from an early age (Shehzad *et al*., 2009; Barro-Kondombo *et al*., 2010; Jugran *et al*., 2013). Furthermore, Duminil and Michele (2009) reported that the principal character used for identification and description of plant taxa has been morphological features and properties. Therefore, the evaluation of the diversity and its nature and significance are beneficial or even essential to a breeding program (Lacis *et al*., 2010). High level of genetic variability was observed in *Argania spinosa*, which has not yet been well explored and exploited, ecotypes are expected to develop. So, previous studies based mainly on agro-morphological traits have revealed that there is high variation level of phenotypic characters in flowers, leaves, fruits and seeds (Ait Aabd *et al*., 2011; Bani Aameur and Ferradous, 2001; Ferradous, 1995; Zahidi, 1997).

Breeding programs for stone fruit trees have mostly designed on the main tree, nut and kernel properties, such as bud-break time, and male and female flowering date, bearing habit, kernel weight, kernel color and also kernel percentage (Khadivi-Khub and Ebrahimi, 2015; Arzani *et al*., 2008). However, despite of the reports about argan tree, we still do not have comprehensive information about the phenotypic and genetic diversity of this wild species. Therefore, this study aims to describe and evaluate morphological variation of *Argania spinosa* trees, determine the correlation among traits, identify elite individuals among several populations collected from different regions of Morocco for breeding programs. In this line the useful tools for characterization of the plant genetic resources are morphological characterization combined with multivariate statistical methods, like Principal Component Analysis (PCA) and cluster analysis (Martínez-Calvo *et al*., 2008). Multivariate techniques can help to evaluate large data sets and resolve several phenotypic and genotypic measurements into fewer more interpretable and more easily visualized groups. PCA is a way to visualize the differences between individuals, identifying possible groups and relationships between individuals and the variables (Martínez-Calvo *et al*., 2008). Also, cluster analysis enables analyzing simultaneously both quantitative and qualitative traits, and each entry is treated as an individual entity of equal weight (Peeters and Martinelli, 1989; Iezzoni and Pritts, 1991).

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Materials and methods

Plant material and study area

This study is conducted in different distribution areas of the argan tree (Oujda, Rabat and South West), six hundred and eightieth native trees are selected for all regions according to high yield and the information elicited from the rural population (table 1). The selected trees were healthy and mature. The sampled individuals were chosen in order to provide the maximum representation of the ecological conditions of the areas.

Table 1 - Regions and sample size of studied plant material

Regions	Total	Rabat		Oujda	South West					
		Oued Charrat	Oued Grou	Beni Snassen	Tagadirt	Amskroud	Toufaline	Essaouira	Guelmim	Agadir
Tree number	680	71	94	77	29	30	15	120	20	224

Sampling and morphological characterization

Morphological characterization of each tree was performed according to agromorphological descriptors of fruit and forest species due to the absence of specific descriptors of argan tree. In this work, sixteen morphological and agronomic characteristics were used to estimate the range of morphological variation among argan trees. Ten fruits and leaves were evaluated from each tree then the mean was calculated. In total 6800 leaves and fruits were characterized. All fruits used in this study were collected at mature stage. Morphological traits were split into qualitative and quantitative traits.

On a large scale, the qualitative traits are more credible and simple to use for the morphological variability studies. Qualitative characters were noted by a general visualization. A total of seven qualitative traits were considered: Tree branching, fruit color, fruit shape, leaf color, spine, growth habit, fruit-spine (table 2).

Quantitative characters are traits for which phenotypic variation is continuously distributed in natural populations, with population variation often approximating a statistical normal distribution on an appropriate scale. The quantitative traits were subdivided into two groups: traits related to the tree and leaves and traits related to the fruits. The measured characters were: Tree height (m), first leaf height from the soil (m), trunk diameter (cm), branches number, crown diameter (distance between the two opposite edges of the crown) (m), fruit length (cm), fruit width (cm), leaf length (cm) and leaf width (cm).

Table 2 - Codes and description of the qualitative variables used for the characterization of studied individuals

CHARACTER	STATE
Leaf color	1 = light green; 2 = dark green
Spine	1 = not thorny; 2 = very little thorny; 3 = little thorny; 4 = moderately thorny;5 = thorny; 6 = very thorny; 7 = very very thorny
Tree branching	1 = not branched; 2 = little ramified; 3 = ramified; 4 = very ramified; 5 = very very ramified; 6 = very very very ramified
Growth habit	1 = tree erect ; 2 = tree semi erect ; 3 = weeping tree
Fruit color	1 = yellow; 2 = yellow green; 3 = light green; 4 = dark green 5 = black green; 6 = red yellow; 7 = red; 8 = green brown; 9 = brown; 10 = green red; 11 = green with red spots; 12 = green with small red spots; 13 = green with large red spots; 14 = green with very red spots
Fruit shape	1= round; 2 = pear; 3 = oval; 4 = extended; 5 = long; 6 = oblong; 7 = fusiform; 8 = round-oval; 9 = round-extended; 10 = round-long; 11 = oval-elongated
Fruit-spine	1 = absent; 2= present

Data analysis

Descriptive statistics such as averages and Coefficients of Variation (CV) were used to detect diversity between and within the studied individuals , Following correlation analysis, using Pearson correlation coefficient (α = 0,01), was used to estimate the relationships between studied variables. Principle component analysis (PCA) was used to detect the characters that are most relevant to distinguish between trees , Clusters of trees were formed on the basis of these factors using Ward's method; the graph representing classification is a dendrogram of dissimilitude with the proportion of variance accounted for by the cluster (R square). Statistical analyses were carried out using SAS (Statistical Analysis Software).

Results

Contents variability of morphological traits

Description of phenotypic traits is greatly important during the domestication and introduction of fruit species. The information about the analyzed variables in 680 trees, including mean value, variability range, variation coefficient, and minimum significant difference among trees, is reported in table 3. In most traits under study, there is a good diversity in this set of individuals, among the characters measured, branches number showed the highest coefficient of variation (CV =89. 76 %), while the lowest CV was related to fruit length CV = (22.00%).

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Table 3 - Descriptive statistics for quantitative morphological traits among studied Argania spinosa individuals.

VARIABLE	N	MEAN	STANDARD DEVIATION	MINIMUM	MAXIMUM	COEFFICIENT OF VARIATION
Crown diameter (m)	680	6,09	2.87	0.50	18.00	47.13
Tree height (m)	680	6.39	3.12	1.00	20.00	48.88
Trunk diameter (cm)	680	27.77	20.70	18.00	60.00	74.56
First leaf height (m)	680	2.01	1.39	0.00	9.33	69.05
Branches number (cm)	680	3.84	3.45	0.00	26.00	89.76
Fruit width (cm)	680	1.82	0.50	0.50	4.64	27.70
Fruit length (cm)	680	2.81	0.62	0.83	5.50	22.00
Leaf width (cm)	680	0.62	0.24	0.26	2.68	38.86
Leaf length (cm)	680	2.43	0.65	0.77	5.70	26.83

Correlation between quantitative traits

Correlation is a measure of the degree to which variables vary together or a measure of intensity of association. Correlation coefficients displayed significant results for various quantitative traits (Table 4). The coefficient of correlation resulted low for all the observed pairs of variables. A negative and statistically significant correlation was observed between the trunk diameter and number of branches (r=-0.18), as well as between the first leaf height and the length (r=-0.33) and width of fruit (r=-0.26). Indeed, the probability of having large fruits increases with the decrease of the first leaf height distance. Positive and statistically significant correlations observed between leaf parameters and fruit parameters varied between r= 0.15 and r= 0.48.

Table 4 - Correlations coefficient among measured variables for all studied argan trees

	CROWN DIAMETER	TREE HEIGHT	TRUNK DIAMETER	FIRST LEAF HEIGHT	BRANCHES NUMBER	FRUIT WIDTH	LEAF LENGTH	LEAF WIDTH	LEAF LENGTH
Crown diameter	1.00								
Tree height	0.31 <.0001	1.00							
Trunk diameter	0.29 <.0001	0.18 0.0001	1.00						
First leaf height	0.21 <.0001	0.34 <.0001	0.13 0.0051	1.00					
branches number	0.09 0.09	-0.02 0.72	-0.18 <.0001	-0.09 0.05	1.00				
Fruit width	0.03 0.68	0.20 0.0004	0.10 0.07	-0.26 <.0001	0.02 0.70	1,00			
Leaf length	-0.0036 0.96	0.17 0.0031	0.07 0.22	-0.33 <.0001	-0.03 0.62	0.48 <.0001	1.00		
Fruit width	-0.0034 0.95	0.14 0.0088	0.08 0.10	0.07 0.20	-0.01 0.81	0.15 0.0039	0.15 0.0036	1.00	
Leaf length	0.13 0.02	0.13 0.02	0.23 <.0001	0.11 0.05	0.04 0.50	0.22 <.0001	0.22 <.0001	0.39 <.0001	1.00

The results of correlation of leaf and fruit parameters were low but they were high between the characteristics of the same organ. Moreover, display of the tree showed positive association with trunk diameter, the first leaf height, plant height and leaf length. Tree height also showed positive correlation with trunk diameter, the first leaf height, fruit length, fruit width and leaf length.

Distribution percentage of qualitative traits

For qualitative characteristics, the distribution percentage shows great variability for each trait (table 5). In fruit, the most abundant shape and color are the light green and elongated shape with a percentage respectively 44.43% and 52.92%. The less abundant fruit traits are round–long, oval-stretched, oblong and pear shaped for the shape (0.17%) and green, black green, green with red spot for the color (0.19 %). Fruit color is one of more important traits in the selection program, it tells us about the stages of development and the tree precocity. The frequencies in the table reveal that thorny and moderately thorny trees are the most common with 41-42%. One of the important purposes in argan breeding is selecting the least thorny trees to facilitate harvesting. In this work, the highest percentage of architecture is observed in individuals moderately branched (53%). For the growth habit, the upright shape presents 50% versus 12.5 % for the semi upright and 37.5% for the weeping.

Table 5 - Distribution percentage for qualitative characteristics: The numbers from 1 to 14 correspond to scale defined for each qualitative character

Characteristics	Scale													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Fruit color	12.24	8.85	3.77	52.92	0.19	3.01	3.20	0.19	1.13	8.10	2.45	2.07	1.69	0.19
Fruit shape	23.65	0.17	19.93	44.43	2.20	0.17	7.94	0.84	0.34	0.17	0.17			
Spine	41.06	0.38	12.55	42.02	2.28	1.52	0.19							
Tree branching	25.17	5.49	53.09	15.33	0.69	0.23								
Growth habit	50.00	12.50	37.50											
Leaf color	99.67	0.33												
Fruit-spine	59,52	40.48												

For each qualitative descriptor the frequency of different values was calculated. Significance of these descriptors was tested using the Chi Square analysis. Chi square test have shown significant differences in following qualitative traits at an alpha level of 0.05 and p <. 0001 (table 6):

The frequency distribution of the seven qualitative descriptors indicated homogeneity of distribution in six traits, whereas significant differences were observed in fruit color (< 0.001), fruit shape (< 0.001), leaf color (< 0.001), tree branching (< 0.001), presence of spine (< 0.001), growth habit (0.0009).

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Table 6 - Chi-square test for comparison of frequency distribution for seven qualitative traits

TRAITS	CHI SQUARE TEST FOR EQUAL PROPORTIONS	
Fruit color	χ^2	1804.94
	DF	13
	Pr>ChiSq	<.0001
Fruit shape	χ^2	1360.95
	DF	10
	Pr>ChiSq	<.0001
Spine	χ^2	805.68
	DF	6
	Pr>ChiSq	<.0001
Tree branching	χ^2	537.81
	DF	5
	Pr>ChiS	q<.0001
Growth habit	χ^2	14.00
	DF	2
	Pr>ChiSq	0.0009
Leaf color	χ^2	596.03
	DF	1
	Pr>ChiSq	<.0001
Fruit-spine	χ^2	3.05
	DF	1
	Pr>ChiSq	0.0809

Principal component analysis

Principal Component Analysis (PCA) was used to decrease the number of initial variables, description and explanation of total diversity in a population and determination of each trait's contribution to total variation are used (Pearson, 1901). PCA revealed that the first four axes account for 65.6% of the variation of the traits measured in the 177 trees. The first four components were retained as per Kaiser criterion (Kaiser, 1960). This criterion retains only the components that have eigenvalues greater than 1 for interpretation. Loading variables and the PCA scores were also calculated (Table 7 and 8).

Table 7 - Eigenvalues, individual and cumulative percentages of variation explained by the first four principal components (PC)

AXES	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
1	1.99	0.42	0.22	0.22
2	1.57	0.23	0.17	0.39
3	1.33	0.33	0.15	0.54
4	1.00	0.02	0.11	0.65

Table 8 - Eigenvectors of four main principal components (PC) explaining 65% of total variation of the 177 studied argan individuals

Character	PC1	PC2	PC3	PC4
Crown diameter	-17	4	17	83 *
Tree height	11	15	81 *	10
Trunk diameter	44 *	-8	9	62 *
First leaf height	-9	-1	84 *	12
Branches number	-62 *	37	-10	17
Fruit width	69 *	17	5	2
Leaf length	73 *	29	-14	12
Fruit width	15	75 *	25	-21
Leaf length	7	83 *	-2	12

The PC1 explains 22.1% of total variation. It combines positives values of length and width of fruits, trunk diameter and negative value of branches number, the second PC2 accounting for 17.5% of total variation was mainly composed of length and width of leaves. The third principal component axis PC3 which accounted for 14.9% of the observed variation was dominated by trait corresponding to tree height and the first leaf height. PC4 was built from trunk diameter, tree display and accounted for 12% of the total variability. The configuration of the 177 trees along the first three principal component axes is illustrated in Figure 1. The characters best interacted with the three axes helped us to know the most important morphological traits for an identification and comprehensive description of the studied trees. The ordination of individuals on PC1, PC2 and PC3 which accounted 54 % of total variation revealed that the individuals S220, S233, S277, S305 S309 and S312 from South West, R162, R174, R179 and R195 from Rabat, O337 and O341 from Oujda were distanced from the centroid (Fig. 1) while the rest were close to the centroid.

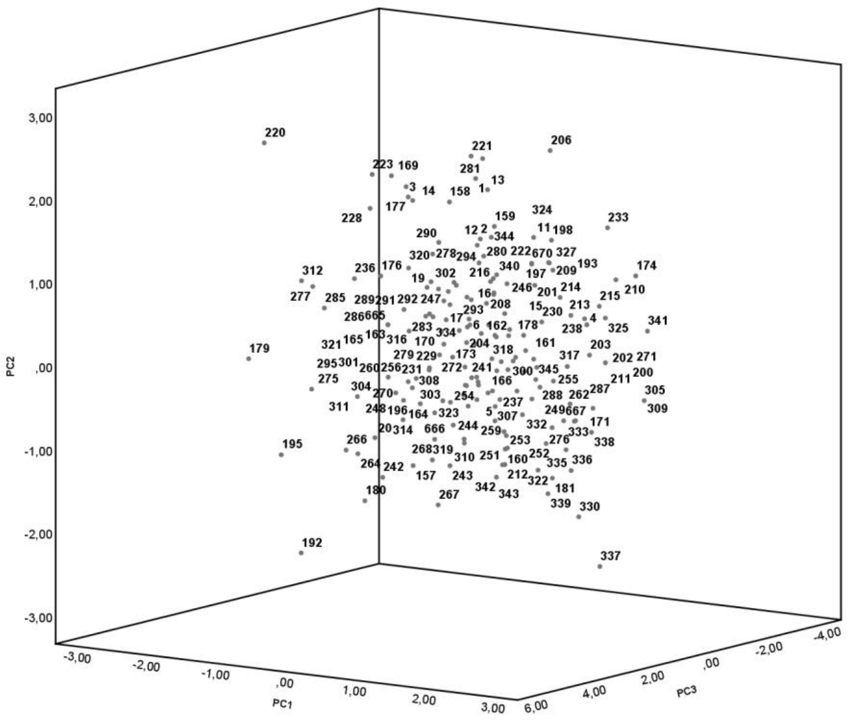

Figure 1 - Scatter plots for the first three principal components (54% of the total variability) for the 177 studied argan individuals based on quantitative phenotypic characters.

Hierarchical cluster analysis

To better appreciate the agro-morphological variability in argan tree, a hierarchical cluster analysis (HCA) was done on the basis of evaluated quantitative characters. In this study, nine of the variables that participate to the global variability illustrated by the axis of the ACP were chosen. Dendrogram based on the measured characters clustered the tree into two clusters (figure 2). The first major cluster I-A containing just two trees out of the 177 individuals studied. The second major cluster I-B consisted of 175 individuals forming two sub-clusters. Sub-cluster II-A possessed two groups, the first group included 31 individuals and the second covered 7 trees and sub-cluster II-B contained the rest of individuals. The individuals collected from different provinces of Morocco were distributed over all clusters, indicating the existence of more diversity. In terms of phenotypic resemblance and morphological characteristics, a scatter plots were prepared based on the PC1 and PC2, PC3 that reflected a relationship among the studied individuals. Results of scatter plot supported the results cluster analysis and genotypes were distributed in the center of plot. The individuals O341 and S305 were detached from others. These trees were characterized by the highest values for trunk diameter and crown diameter.

Discussion

This study shows the level of morphological diversity of argan trees in Morocco. It is the first step in the evaluation of genetic diversity which is very important in breeding process and conservation of plant genetic resources. Results reveal a large degree of differentiation between studied trees. The morphometric analysis of the data between trees shows that fruits and leaves characteristics was the most discriminating descriptors of variability.

Similar studies based on morphological characters have been carried on fruit trees, Ait Aabd (2012) has reported a high diversity between *Argania spinosa* individuals for fruits, seeds and almonds characters studied. Similarly, in the *Vitellaria paradoxa (sapotacea*e) morphological analysis shows a wide variability for traits related to trees, leaves and fruits (Nyarko *et al*., 2012). It is of importance to note that morphological traits of fruits and seeds are the major criteria used in the breeding program, however, the study of the traits related to the tree and the productivity is indispensable in the domestication program. Nonetheless, since the individuals were observed in different regions, the morphological variation that was observed can be attributed to genetic divergence between the tree, age of tree and environment. Our results in some cases were in agreement with those reported by others (Sorkheh *et al*., 2009; Khadivi-Khub and Anjam, 2014; Enaberue *et al*., 2014; Nikoumanesh *et al*., 2011). Enaberue *et al.*(2014) studied fruit, nut and leaf traits in *Vitellaria paradoxa* C,F, Gaertn found that these characters are discriminating for their germplasm. A similar morphological analysis realized by Sorkheh *et al*., (2009) in wild almond species showed that the variables participating to fruit, leaf and tree traits are important in explaining the variation among the selections. In wild species of *Amygdalus subgen*, Nikoumanesh *et al*., (2011) affirmed that the discriminating characters for their germoplasm were correlated to leaf and tree. Thus, leaf, fruit and tree traits variables were consistently presented in the main components and therefore contributed high variability. The goal of PCA is to determine the number of main factors for reducing the number of effective parameters to discriminate individuals. Furthermore, associations between traits emphasized by this method may correspond to a genetic linkage between loci controlling traits or a pleiotropic effect (Iezzoni and Pritts, 1991). A phenotypic variation of argan trees and the correlation between some morphological properties has been demonstrated by other authors (Ait Aabd *et al*., 2012; Zahidi *et al*., 2013).

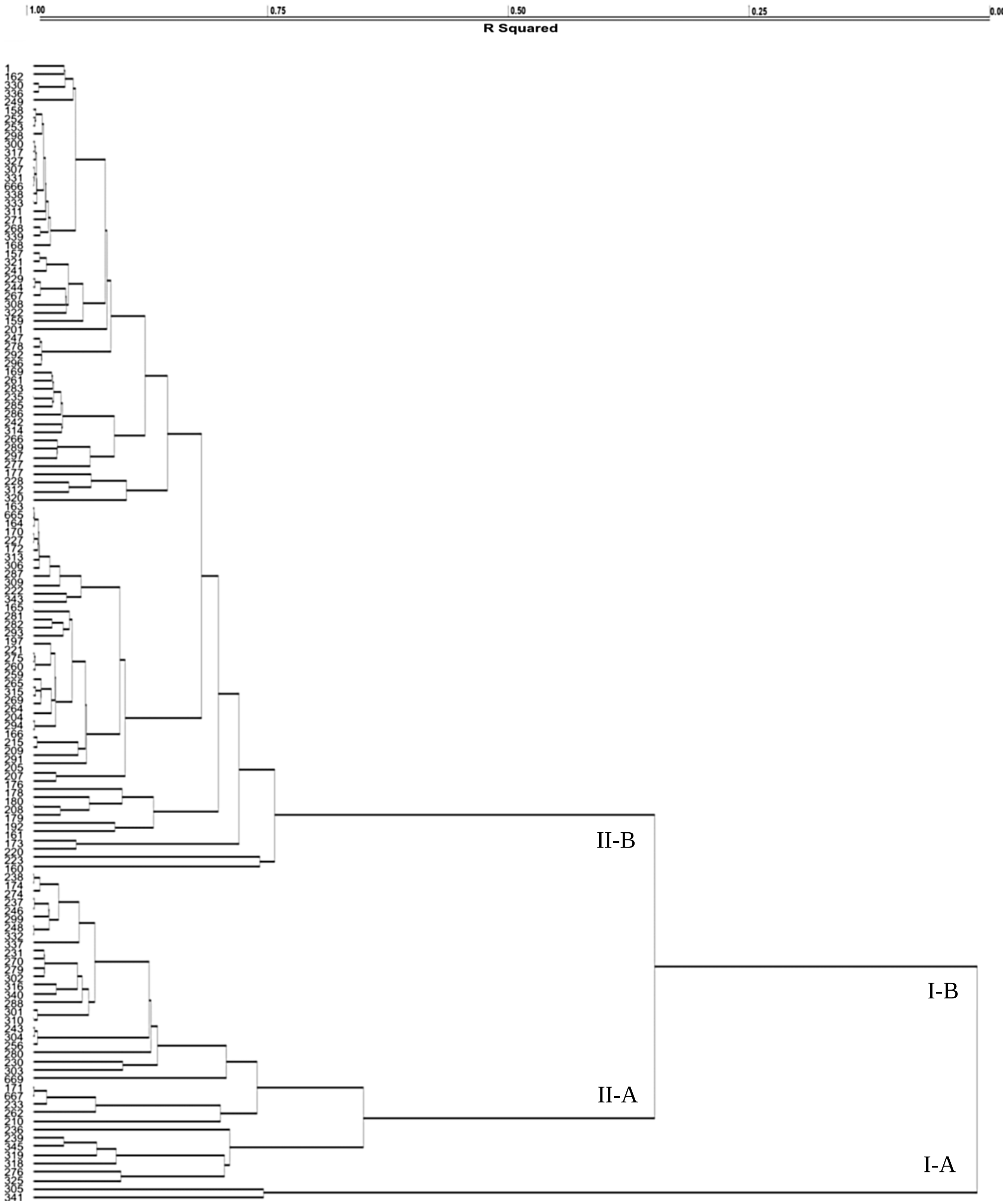

Figure 2 - Cluster analysis of the 177 studied argan individuals based on Ward method estimated from quantitative agro-morphological traits

Significant correlation of leaf and fruit parameters was reported by Kafilatou *et al*. (2015) and Lovett and Haq (2000) when studying the diversity of *Vitellaria paradoxa*. Buba (2013) studied correlation in *Vitellaria paradoxa C,F, Gaertn* and observed that plant height has highly significant and positive association with trunk diameter and crown length as the case in this study. The rate of correlation among traits is so significant that we could find out the changes in relevant trait through measuring another trait; therefore, measurement of a trait could be indirectly done more rapidly and more economically.

Unlike the quantitative parameters, qualitative characters are widely preferred for characterization of the germplasm because they are relatively less influenced by the environment. They form discrete phenotypic classes and therefore are very useful tools in classifying germplasm and can be principally assessed visually and even by novice evaluators. For that, the majority of traits DUS (distinctiveness, uniformity and stability) uses quantitative traits for registration of cultivars (Kruskal, 1978).

The dendrogram and the scatter plots explained the high variation in individuals, revealing that the studied trees were a good candidate gene pool for breeding programs. The difference between cluster and scatter plots can be explained by the variation regarded for the analysis. The HCA is based on the whole variability of phenotypic data, while scatter plot is created according to the cumulative variance explained by the first three principal components. The traits used in this study could be contributed to establish a catalog of argan trees. Additionally, the harmony of multivariable analysis showed that morphological analysis can give reliable information on the variability in argan. Among other woody species and in correspondence with our results, other authors (Nikoumanesh, 2011; Enaberue *et al*., 2014; Khadivi-Khub and Ebrahimi, 2015) showed that morphological evaluation is a useful tool for characterization of tree germplasm and for species distinction. We found no evidence for any relationships in most of the individuals according to their geographic origin, this result was in concordance with the findings found in *Prunus avium* L. (Rakonjac *et al*., 2014). The overall analysis of all traits illustrates a wide diversity that may have important implications for management of the genetic resources.

Conclusion

Agro-morphological characterization of *Argania spinosa* contributes to provide us with a better understanding of the specie. The present study showed that on the basis of agro-morphological traits, there is a wide genetic variation in Morocco argan tree. Morphological characterization of the sixteen traits showed variability within the individuals. Most of the variations are related to the fruit-spine, the branches number and the trunk diameter. The correlation study explicitly revealed the existence of associations among many agro-morphological characters. The multivariate analysis was found useful in determining the phenotypic differences among the trees studied. The results of this work can also help breeders to choose the most varied genotypes to begin crossing and breeding programs. These results emphasize the usefulness of morphological traits in breeding programs to make a better use of the argan tree.

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